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Potential of native palm species in Northeast Brazil as hosts for the invasive mite *Raoiella indica* (Acari: Tenuipalpidae)

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Abstract

The red palm mite, Raoiella indica Hirst (Tenuipalpidae), has mainly been registered on palm species (Arecaceae), and its expansion in Brazil has the potential to cause significant negative impact on cultivated as well as native palms. Here, we evaluate the potential of native palms from Northeast Brazil to act as hosts of R. indica. Specifically, we used in situ free-choice and confinement tests, in which sections of palm leaves/leaflets of various species were experimentally infested with R. indica. We tested the following species: Acrocomia aculeata, Acrocomia intumescens, Allagoptera caudescens, Attalea funifera, Attalea oleifera, Bactris acanthocarpa var. acanthocarpa, Bactris ferruginea, Bactris glassmanii, Bactris hirta var. spruceana, Bactris pickelii, Copernicia prunifera, Desmoncus orthacanthos, Desmoncus polyacanthos, Syagrus coronata and Syagrus schizophylla. All of these were compared with the mite's preferred host, the coconut palm, Cocos nucifera. In the free-choice test, both male and female R. indica preferred C. nucifera in comparison to each of the native palms. In the confinement test, we observed significant differences in the survivorship between mites on native palms and those on coconut palms after the second day of infestation. By the fifth day, survivorship of mites on the native palms was almost always significantly lower than on C. nucifera (excepting for C. prunifera). We conclude that, among all the native palms evaluated, only the carnauba palm (C. prunifera) is at risk from R. indica. This result is relevant as this palm is an economically important species in the region.

Keywords Arecaceae · Host range · Red palm mite · Atlantic forest · Caatinga

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Introduction

The red palm mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae), was described from India (Hirst 1924), but is now found in Africa, North, Central and South America, Asia, the Caribbean and the Middle East, where it infests economically important crops such as coconut and, especially in the neotropics, banana and tropical flowers (Peña et al. 2012; Navia et al. 2015). Colonies of *R. indica* develop on the ventral surface of leaves, where mites feed via the stomata (Ochoa et al. 2011), damaging the cells of the leaf mesophyll, and causing a gradual yellowing, followed by tanning and necrosis (Carrillo et al. 2012). Infestation on young plants can lead them to death in nurseries or in the field (Navia et al. 2015).

Since its first report to the Western Hemisphere in 2004 (Flechtmann and Etienne 2004), *R. indica* has spread geographically and dramatically increased its range of host plants from less than 10 (Nageshachandra and Channabasavanna 1984; Flechtmann and Etienne 2004) to more than 100 species (Cocco and Hoy 2009; Carrillo et al. 2012; Gondim et al. 2012; Vásquez and Moraes 2012; Navia et al. 2015; Gómez-Moya et al. 2017). Host families include the Arecaceae, Cannaceae, Cycadaceae, Heliconiaceae, Musaceae, Pandanaceae, Strelitziaceae and Zingiberaceae, with approximately 81% of host species belonging to the family Arecaceae (Carrillo et al. 2012).

The red palm mite has the potential to become a major problem for agriculture in Brazil, given that some hosts are of high economic importance. Brazilian Arecaceae are represented by 300 species, varieties and natural hybrids (Lorenzi et al. 2010), which occupy all the biomes of the country. Many of these palm species are exploited by low-income populations in the North and Northeast regions (Clement et al. 2005; Lorenzi et al. 2010). In addition to exploitation for wax, food and building materials, the enormous phenotypic diversity of palms makes them ideal for landscaping and ornamental uses (Lorenzi et al. 2010).

Most studies on the potential effects of R. indica have focused on Cocos nucifera or other economically important hosts, such as date palm, oil palm, banana, plantain and ornamental plants (Cocco and Hoy 2009; Carrillo et al. 2010; Vásquez and Moraes 2012; Otero-Colina et al. 2016). Even though new hosts have been reported at sites of invasion, about 75% of published studies addressing the potential effects of R. indica in South America refer exclusively to exotic plants (Carrillo et al. 2012). Thus, little is known on the potentially negative impacts of R. indica on native host plants in the neotropics, and more in-depth studies on the biology of this mite on native palms are urgently needed. The limited evidence that exists comes mainly from the Amazon region. For example, Gondim et al. (2012) reported the occurrence of R. indica on five palm species native to the Brazilian Amazon. Similarly, Gómez-Moya et al. (2017) identified four species of native Amazonian palms as hosts and three as secondary hosts. However, to our knowledge nothing has been published about the host potential of native palms from other Brazilian biomes, such as Atlantic forest, cerrado and caatinga. The Northeast region of Brazil is particularly rich in palms, many of which are exploited at low scale by local populations. For example, Lyra-Lemos (1987) documented the occurrence of 22 native species of Arecaceae in the Northeast state of Alagoas. These species range from the coast to the semi-arid interior, passing through perennial coastal forest, subperenifolia forest, subcaducifolia forest, and caatinga vegetations.

As *R. indica* continues to expand its geographic distribution in the neotropics, including within Brazil (Marsaro et al. 2018; Melo et al. 2018), it is vital that scientists assess the risks of plant species to become hosts. Thus, the aim of the present study was to evaluate



the potential of native palms from Northeast Brazil as host plants to the invasive red palm mite.

Material and methods

Palms and mites

Fifteen species of palms native to Northeast Brazil (Table 1) were studied. Plant material (leaves/leaflets) of native palms and coconut palm was collected in various sites in the State of Alagoas, Brazil. Leaves and/or leaflets of the palms were cut with pruning shears, placed in plastic bags, packed in thermal boxes and transported to the laboratory to be used on the same day. The leaves were kept with their bases soaked in a vessel with water for approximately 2 h for rehydration prior to infestation. In addition, inflorescences and fruits were collected, according to their availability, and taken to the herbarium of the Instituto do Meio Ambiente do Estado de Alagoas (Herbário MAC) where exsiccatae were prepared, species identification was confirmed, and vouchers were deposited (Table 1).

Specimens of *R. indica* were collected in a naturally infested pygmy date palm *Phoenix roebelenii*, from a residential garden in the city of Maceió, AL, Brazil. The infested leaflets were collected as described above and transported to the laboratory for immediate use.

Free choice test

The experimental unit was composed of one leaflet section (5 cm long) of a native palm species arranged perpendicular to one *C. nucifera* palm leaflet section, both with the abaxial surface facing upwards, in a Petri dish (14 cm diameter), on a soaked polyurethane sponge disc to maintain turgidity and avoid mite escape. Males and females were tested separately to avoid interference caused by attraction of the sexes to each other. Each section was infested with five active adults of *R. indica*. The dishes were kept in 'biological oxygen demand' (B.O.D.) chambers at 25 ± 1 °C, $70 \pm 10\%$ relative humidity and 12-h photophase. Under stereomicroscope, the number of individuals present in each section was evaluated daily, during 8 days, or while there were live mites. Only living individuals were counted, and non-quiescent mites that did not move when gently touched with a fine brush were considered dead (Stark et al. 1997). As a control, a blank treatment was also performed, composed of two sections of coconut leaflets, in the same conditions previously described.

Confinement test

The leaflets of each species were sectioned into 5-cm-long fragments and arranged with the abaxial surface upwards, in an experimental unit composed of a Petri dish (14 cm diameter), on polyurethane sponge disks moistened to maintain turgidity. Leaflet sections of *C. nucifera* were used for comparison, as this species is a preferential host for *R. indica* (Gondim et al. 2012; Kane et al. 2012; Otero-Colina et al. 2016). Each section was infested with five pairs (males harboring freshly emerged females) of adult *R. indica* and kept in a B.O.D. chamber at 25 ± 1 °C, $70 \pm 10\%$ RH and 12-h photophase. Under stereomicroscope, the following parameters were evaluated on a daily basis for 18 days or while living mites were present: (i) number of live adults, (ii) number of eggs, and (iii) number



Table 1 Native palm species from Northeast Brazil evaluated for host suitability to Raoiella indica, voucher numbers, their common names, distribution within Brazil and uses (based on Lyra-Lemos 1987; Lorenzi et al. 2010 and Flora do Brasil 2020 em construção 2019)

Palm species	Vouchera	Common names	Voucher ^a Common names Distribution within Brazil		Uses ^{d,e}
			States ^b	Phyto- geographic domains ^c	
Acrocomia aculeata (Jacq.) Lodd. ex Mart	62159	Macaúba	BA, CE, ES, GO, MA, MG, MS, MT, PA, PI, RI, SP, TO	Af, Ce	cn (s), fi (l), hf (m), or
Acrocomia intumescens Drude	65158	Macaúba	AL, BA, CE, PB, PE	Af	hf (m), or
Allagoptera caudescens (Mart.) Kuntze	65147	Buri	AL, BA, ES, RJ, SE	Af	hf (h), or
Attalea funifera Mart. ex Spreng	65063	Piaçava	AL, BA, SE	Af	cn, cw (f), fi, hf (a), or
Attalea oleifera Barb. Rodr	65148	Pindoba	AL, BA, ES, MG, PB, PE, SE, SP	Af, Ce	cn (l), cs (m), fu (m), hf (a), or
Bactris acanthocarpa Mart. var. acanthocarpa	65149	Tucum	AC, AL, AM, BA, ES, MA, PA, PE, RO, SE	Af, Am	af (f), or
Bactris ferruginea Burret	65150	Coco-de-fuso	AL, BA, ES, MG, PE, SE	Af	fi (l), hf (f)
Bactris glassmanii MedCosta & Noblick ex A.J.Hend	65062	Marajá	AL, BA, ES, PE, SE	Af	hf (f), or
Bactris hirta Mart. var. spruceana (Trail) A.J.Hend	65151	Marajazinho	AL, AM, AP, BA, ES, MA, PA, PE, RO, RR, SE	Af, Am	or
Bactris pickelii Burret	65152	Tucum-mirim	AL, BA, ES, PB, PE, SE	Af	or
Copernicia prunifera (Mill.) H.E.Moore	65061	Carnaúba	AL, BA, CE, MA, MG, MT, PB, PE, PI, RN, SE, TO	Ca, Ce	af (l), cn (s), cw (l), or, wa (l)
Desmoncus orthacanthos Mart	65153	Titara	AC, AL, AM, AP, BA, ES, MA, PA, PB, PE, RI, RO, RR, SE	Af, Am	af (f), cw (s), or
Desmoncus polyacanthos Mart	65154	Titara	AC, AL, AM, AP, BA, CE, ES, GO, MA, MG, MT, PA, PB, PE, RJ, RN, RO, RR	Af, Am, Ce	cw (s), or



Table 1 continued

Palm species	Voucher	Common names	/ouchera Common names Distribution within Brazil		Uses ^{d,e}
			States ^b	Phyto- geographic domains ^c	
Syagrus coronata (Mart.) Becc	65156	65156 Ouricuri	AL, BA, MG, PE, SE	Ca, Ce	cs (a), cw (e), hf (a, m, h), wa (l), or
Syagrus schizophylla (Mart.) Glassman	65157	Aricuriroba	AL, BA, ES, PE, SE	Af	hf (m), or

^bBrazilian states—AC Acre, AL Alagoas, AM Amazonas, AP Amapá, BA Bahia, CE Ceará, ES Espírito Santo, GO Goiás, MA Maranhão, MG Minas Gerais, MS Mato Grosso do Sul, MT Mato Grosso, PA Pará, PB Paraíba, PE Pernambuco, PI Piauí, RJ Rio de Janeiro, RN Rio Grande do Norte, RO Rondônia, RR Roraima, SE Sergipe, SP São Paulo, ^aDeposited in the herbarium of the Instituto do Meio Ambiente do Estado de Alagoas (Herbário MAC)

Phytogeographic domains—Af Atlantic forest, Am Amazon, Ca caatinga, Ce cerrado

TO Tocantins

^dUses—qf animal feed, cn construction, cs cosmetics, cw craftwork, fi fiber, fu fuel, hf human feed, or ornamental, wa wax

ePlant parts—a almond, e endocarp, f fruit, h palm heart, l leaf, m mesocarp, s stem

of post-embryonic individuals in each leaflet section. Non-quiescent individuals who did not move when gently touched with a fine brush were considered dead (Stark et al. 1997). Every 5 days, the sections were replaced with new ones, to maintain an adequate nutritional condition of the substrate. Each native species was compared to *C. nucifera* leaflets, infested and maintained under the same conditions.

Experimental design and statistical analysis

Both experiments followed the completely randomized experimental design. The free choice test was composed of 15 treatments and 10 replications. Each combination of native species×coconut was considered as one treatment and each experimental unit with a pair of leaflet sections was considered as one replication. Choice data were submitted to frequency analysis and evaluated by the χ^2 test at 1% of probability using the GENES software (Cruz 2013, 2016).

The confinement test was composed of 16 treatments (15 native palm species + C. nucifera) and 10 replications, each plate containing a leaflet section being considered a replication. The averages were submitted to analysis of variance and survivorship and oviposition on the palms in the same day were compared by the Scott-Knott test at 5% significance, using the GENES software (Cruz 2013, 2016). Raw data were transformed by $\sqrt{(x+0.5)}$ in order to normalize data distributions for statistical analyses.

Results

Free choice test

Both males and females of R. indica significantly preferred C. nucifera in relation to the native palm in all the evaluation days (χ^2 expected=6.635, P<0.01) (data not shown). The same pattern was maintained when all individuals were considered, regardless of sex (Fig. 1). The preference for C. nucifera was gradually manifested after infestations. In most native palms, between the 4th and 5th days of evaluation, almost 100% of the R. indica individuals had manifested their preference for C. nucifera (data not shown).

Confinement test

There was no *R. indica* mortality in any of the palms on the 1st day after infestation (Fig. 2). Significant differences between survival on native palms and *C. nucifera* (100% survival) were observed from 2nd day for *A. aculeata* (91 \pm 4.1%) (F=4.89); from 3rd day for *B. hirta* var. *spruceana* (95 \pm 1.7%), *B. ferruginea* (93 \pm 1.5%), *B. pickelli* (91 \pm 1.0%), *A. caudescens* (90 \pm 0.0%), *A. funifera* (90 \pm 0.0%), *A. oleifera* (90 \pm 0.0%), *D. orthacanthos* (90 \pm 0.0%) and *D. polyacanthos* (87 \pm 3.0%) (F=16.0); from the 4th day for *B. acanthocarpa* var. *acanthocarpa* (94 \pm 1.6%) and *S. coronata* (93 \pm 1.5%) (F=54.93); from 5th day for *B. glassmanii* (93 \pm 1.5%), *S. schizophylla* (91 \pm 1.8%) and *A. intumescens* (65 \pm 2.2%) (F=57.71); and from the 10th day for *C. prunifera* (76 \pm 2.2%) (F=191.04, all P<0.05). From the 5th day, survival on almost all native palm species was statistically lower than on *C. nucifera*, except for *C. prunifera*. Mortality on *C. nucifera* started on the 5th day, with 99.6 \pm 0.22% survival and, on *C. prunifera*, started on the 8th day, with 95 \pm 2.2% survival. Total mortality of the individuals (0% survival) was observed on



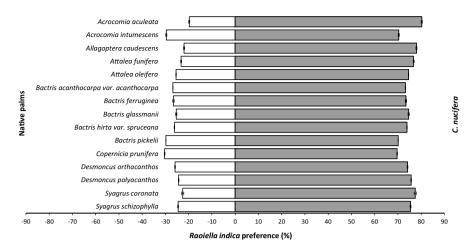


Fig. 1 Mean (±SE) preference (%) of *Raoiella indica* adults by coconut palm *Cocos nucifera* vs. each of 15 native palm species from Northeast Brazil, in a free choice test (average of 8 days, with 10 replications/day)

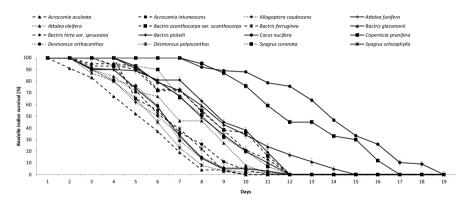


Fig. 2 Mean (±SE) survival (%) of *Raoiella indica* on coconut palm *Cocos nucifera* and 15 native palm species from Northeast Brazil (untransformed data)

A. aculeata, B. ferruginea and D. polyacanthos by the 10th day; on A. intumescens by the 11th day; on A. caudescens, A. funifera, A. oleifera, B. acanthocarpa var. acanthocarpa, B. hirta var. spruceana, B. pickelli, D. orthacanthos and S. coronata, by the 12th day; on B. glassmanii by the 15th day; and on C. prunifera by the 17th day after infestation, when there was no longer survival on any of the native palms. By the 19th day of the experiment there were no adult survivors on C. nucifera.

On the 1st day after infestation, there was no oviposition of R. indica on any of the treatments. During the whole evaluation period, oviposition was observed only on three native species (C. prunifera, S. coronata and S. schizophylla) and on C. nucifera. On S. coronata there were two eggs on the 2nd day and on S. schizophylla one egg on the 3rd day, with 0% viability for both (data not shown). The oviposition of R. indica on C. nucifera started from the 2nd day and ranged from $0.14 (\pm 0.04)$ to $1.20 (\pm 0.05)$ eggs/female/day. On C. prunifera it started from the 3rd day, ranging from $0.18 (\pm 0.07)$ to $0.42 (\pm 0.11)$ eggs/female/day (Fig. 3). The number of eggs/female/day on C. nucifera differed from all native palms



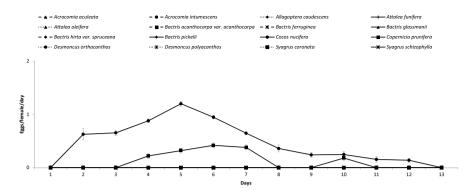


Fig. 3 Mean (±SE) oviposition (no. eggs per female per day) of *Raoiella indica* on coconut palm *Cocos nucifera* and 15 native palm species from Northeast Brazil (all females developed prior to the experiment on *Phoenix roebelenii*) (untransformed data)

in all days with oviposition (P < 0.05). In the 4th, 5th, 6th, 7th and 10th days, oviposition on C. prunifera also differed from the other native palms, and from C. nucifera. Daily and total oviposition differed significantly among C. nucifera (0.55 ± 0.01 eggs/female/day and 6.09 ± 0.13 eggs/female, respectively), C. prunifera (0.22 ± 0.09 eggs/female/day and 1.52 ± 0.10 eggs/female, respectively), and all other native palms (0.0 - 0.003 eggs/female/day and 0.0 - 0.04 eggs/female, respectively) (F = 2068 and 2114, respectively) (data not shown).

Discussion

Mites eventually walked on the underside of the leaflets when they bent, but there was no escape or drowning. The red palm mite always preferred sections of coconut palm in our free choice test. This corroborates the common observation that *C. nucifera* is the preferential host for *R. indica* (Gondim et al. 2012; Kane et al. 2012; Otero-Colina et al. 2016).

The inability of R. indica to survive and reproduce on most native palm species from Northeast Brazil may be associated with its lack of adaptation to the palm's defense mechanisms. Morphological barriers, such as toughness of the cuticle, thickness of the epidermis or some other tissue of the plant, and the presence of chemical deterrents (e.g. terpenoids, flavonoids, tannins, alkaloids, etc.) to feeding and/or oviposition may be sufficient to prevent the native palms from being infested. The variability of the leaf structure may also present problems for mite infestation. The palms are characterized by distinct mechanical or physiological characteristics, such as the scales of the abaxial leaf surface, the presence of epicuticular wax, and the presence of trichomes in various densities, among others (Wittstock and Gershenzon 2002; Vendramim and Guzzo 2009, 2011; Beard et al. 2012). These morphological factors can influence pest behavior and physiology. The former by impairing their locomotion, mating and host selection for feeding and oviposition. Physiological effects may result from the influence of the palm on food intake and digestion, for example, or from the low nutritional value of the host material (Vendramim and Guzzo 2009, 2011; Vendramim et al. 2019). In the specific case of R. indica, stomatal morphology is an important host characteristic (Beard et al. 2012), since this mite inserts its stylets through these openings to feed. Anatomical cuts of the species A. aculeata and S. coronata



indicate a unisseriate epidermis with a thick cuticle (Oliveira 2014), which may be a barrier for penetration of the mite's stylets into the stomatal cells.

The results of our confinement test corroborated previous observations of *R. indica* on *C. nucifera* under laboratory conditions, where oviposition varied from 1 to 6 eggs/female with an average of 2 eggs/female/day (Moutia 1958) and 0.8 eggs/female/day (Vásquez and Moraes 2012). However, under greenhouse conditions, Gómez-Moya et al. (2017) observed 15.4 eggs/female in *C. nucifera*, with an oviposition period of 18.6 days. Nageshachandra and Channabasavanna (1984) recorded 22 eggs/female and a mean oviposition period of 46.9 days for fertilized females and 40.07 days with parthenogenetic reproduction. The observed variation in reported results is probably because studies have been conducted at temperatures that range between 15 and 29 °C (Fidelis et al. 2019). Gómez-Moya et al. (2017) also suggested that the development of *R. indica* is negatively affected when it is fed with detached plant leaves as these may close their stomata interfering with mite feeding (Beard et al. 2012). However, Fidelis et al. (2019) showed that, at least for *Adonidia merrillii*, leaf detachment does not affect the performance of *R. indica*, supporting the reliability of our results.

With the exception of *C. prunifera*, *R. indica* was not able to increase its population on the native palms evaluated in the confinement bioassay, indicating their probable inability to exploit these species under field conditions. Similarly, the native palms *Sabal palmetto* and *Serenoa repens* were observed to be unsuitable hosts for *R. indica* in Florida, USA (Carrillo et al. 2012). These results also corroborate those presented by Cocco and Hoy (2009), with the same native palms and *Sabal minor* in Florida, where *R. indica* females did not survive on leaf discs. In Brazil, Gómez-Moya et al. (2017) studied 12 species of Arecaceae native to the Amazon in greenhouse conditions, for 28 days. Four of these species (*Astrocaryum jauari*, *Bactris simplicifrons*, *Mauritia flexuosa* and *Socratea exorrhiza*) were considered primary hosts that would be negatively impacted by the expansion of *R. indica* in Brazil. Chronic mortality, associated with low reproduction, is usually indicative of malnutrition (Vendramim and Guzzo 2009, 2011; Vendramim et al. 2019), which may have been caused by non-feeding of mites on the native species evaluated in the present work, with the exception of *C. prunifera*. This indicates the possible occurrence of deterrent substances (rather than toxic ones) in the palm species evaluated.

Arecaceae are important components in the ecology of tropical vegetation and, in the Amazon, for example, are considered sentinel plants for conservation (Goulding and Smith 2007). Besides their enormous ecological value, native palms in Northeast Brazil have considerable socioeconomic importance. Some of them are already domesticated and commercially exploited (such as *A. funifera* and *C. prunifera*), whereas others are harnessed mainly by indigenous and poor communities, who use them in several different ways (Table 1). *Bactris acanthocarpa*, *B. hirta* and *D. polyacanthos* are also very important in other regions, such as the Colombian Amazon, where they are used by indigenous peoples for human and animal consumption, utensils and tools and construction materials, in addition to cultural uses (Mesa and Galeano 2013). With the exception of *C. prunifera*, we generated no evidence that the native palms from the Brazilian Northeast evaluated in the present study have the potential to serve as hosts for *R. indica*. However, it is possible that *R. indica* may adapt to these and other new host palms.

The only native palm evaluated in the present study that appears to be susceptible to *R. indica* infestation was the carnauba palm (*C. prunifera*). These results corroborate our unpublished field observations that this palm species can sometimes be infested by the red palm mite. The genus *Copernicia* originated in Cuba, and *C. prunifera* is one of two Brazilian species of this genus. It grows naturally in Northeast Brazil where it has considerable



socioeconomic importance, being used for landscaping in urban areas, building materials, livestock feed and as a raw material for craftwork (Lorenzi et al. 2010). However, the main product is the carnauba wax, which forms a layer that covers the leaves. Due to its unique properties carnauba wax is considered as one of the most important vegetal waxes, being employed in a wide range of products such as furniture polish, cosmetics, pharmaceuticals and food (Freitas et al. 2019).

In contrast to the other native palms we evaluated, *C. prunifera* is already threatened by the expansion of *R. indica* in Brazil. This is of great concern because not only is *C. prunifera* endangered itself, but it might also serve as a population reservoir for *R. indica*. Our findings also reinforce the importance of knowing the characteristics of the host plants and how they respond to infestation. These observations suggest that the evolution of the *R. indica* distribution in the neotropics is a complex process that needs further investigation to determine the potential threats that this exotic species could cause to native palm species and crops of socioeconomic and ecological importance.

In conclusion, the following native palms from Northeast Brazil are not currently susceptible to infestation by the red palm mite (*R. indica*): *A. aculeata*, *A. intumescens*, *A. caudescens*, *A. funifera*, *A. oleifera*, *B. acanthocarpa* var. *acanthocarpa*, *B. ferruginea*, *B. glassmanii*, *B. hirta* var. *spruceana*, *B. pickelli*, *D. orthacanthos*, *D. polyacanthos*, *S. coronata* and *S. schizophylla*. However, the palm *C. prunifera*, also native to Northeast Brazil, is a suitable host for *R. indica* and may therefore be threatened by the spread of this pest. The association between *R. indica* and *C. prunifera* is reported here for the first time.

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Author contributions IVS and ECG planned, designed and executed experimental work, performed data analyses and wrote the manuscript. RPLL found and collected native palms, prepared exsiccatae and identified species.

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